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REVIEW ARTICLE

Sugars in crop plants

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Abstract

We review current knowledge of the most abundant sugars, sucrose, maltose, glucose and fructose, in the world's major crop plants. The sucrose-accumulating crops, sugar beet and sugar cane, are included, but the main focus of the review is potato and the major cereal crops. The production of sucrose in photosynthesis and the inter-relationships of sucrose, glucose, fructose and other metabolites in primary carbon metabolism are described, as well as the synthesis of starch, fructan and cell wall polysaccharides and the breakdown of starch to produce maltose. The importance of sugars as hormone-like signalling molecules is discussed, including the role of another sugar, trehalose, and the trehalose biosynthetic pathway. The Maillard reaction, which occurs between reducing sugars and amino acids during thermal processing, is described because of its importance for colour and flavour in cooked foods. This reaction also leads to the formation of potentially harmful compounds, such as acrylamide, and is attracting increasing attention as food producers and regulators seek to reduce the levels of acrylamide in cooked food. Genetic and environmental factors affecting sugar concentrations are described.

Introduction

Sugars are the primary products of photosynthesis and perform multiple roles in plants as energy and carbon transport molecules, hormone-like signalling factors, osmotica and the source of materials from which plants make proteins, polysaccharides, oils and woody materials. The most abundant free sugars in plants are the disaccharides, sucrose and maltose, and the monosaccharides, glucose and fructose. The structures of these sugars are shown in Fig. 1.

Glucose and maltose have a free aldehyde group when in the chain form, while fructose has a free keto group (Fig. 1). The presence of these carbonyl groups means that glucose and fructose can act as reducing agents, for example in the Maillard, Benedict and Fehling's reactions, and hence are known as reducing sugars. The Benedict and Fehling's reactions are used to test for the presence of reducing sugars: both rely on the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) and the formation of a red precipitate of cuprous oxide (Cu_2O). Aldoses such as glucose are oxidised to carboxylates, while ketoses such as fructose are oxidised to hydroxy carboxylates. Maltose, which is a disaccharide formed of two glucose units, is also a reducing sugar, because one of its units can exist in open chain form. However, sucrose, a disaccharide of glucose and fructose, and trehalose, another disaccharide comprising two glucose units, are not, because in these cases the two units are linked by the anomeric carbon and so cannot adopt an open chain structure.

Glucose and fructose are shown in their ring and open chain forms in Fig. 1; these two forms exist in equilibrium. The glucose ring is created when the oxygen on carbon number 5 links with carbon number 1 (the carbon in the aldehyde group) and transfers its hydrogen to the carbonyl oxygen to create a hydroxyl group. Rings of this sort containing five carbon atoms and one oxygen atom are called pyranoses. If the hydroxyl group is on the opposite side of the ring from the CH_2OH group, the ring is called α-glucose, whereas when the hydroxyl group is on the same side as the CH_2OH group the ring is called β-glucose. The fructose ring structure is created when the oxygen on carbon number 5 links with carbon

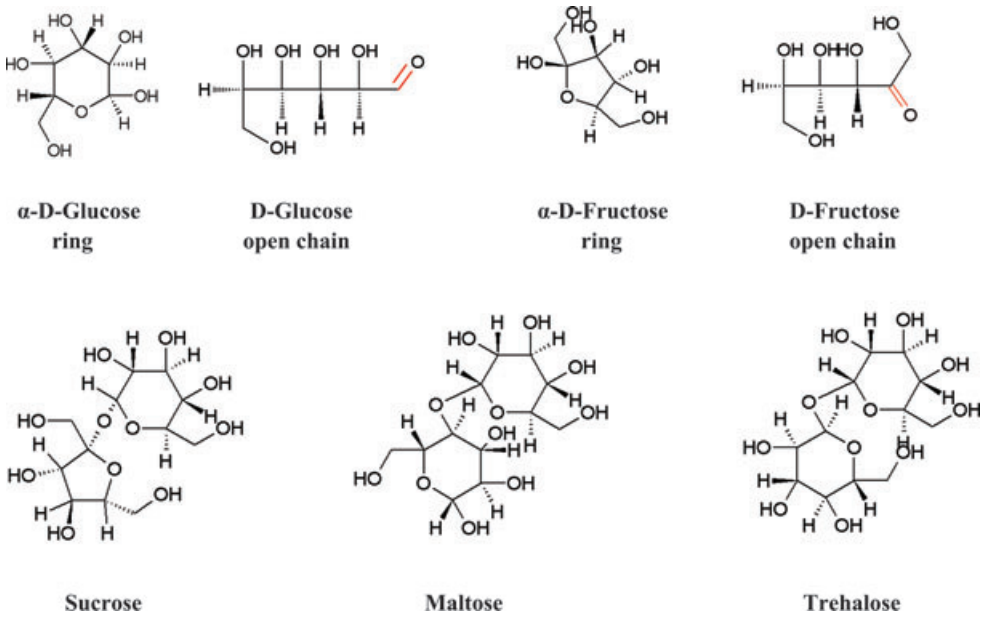

Figure 1 Structures of glucose and fructose, in ring and open chain form, and the disaccharides sucrose, maltose and trehalose. The carbonyl groups of glucose and fructose are shown in red.

number 2 (the carbon in the keto group). Although fructose is a hexose, therefore, it forms a ring with four carbon atoms and one oxygen atom called a furanose. Natural glucose and fructose are also given a D- prefix. Originally this was because they rotate polarised light to the right (dextrorotatory), but it now refers to the absolute configuration of the asymmetric carbon atom furthest from the carbonyl group. The mirror-image levorotary (L-) molecules do not occur in nature.

In this review we consider these key metabolites from the perspective of crop science: their synthesis, their interconversion with polysaccharides, their role as signalling molecules, their participation in the Maillard reaction, with effects on flavour, colour, and the formation of undesirable contaminants, such as acrylamide, and the factors that affect their accumulation. We include the sucrose-accumulating crops, sugar beet and sugar cane, but our main focus is potato and the major cereal crops.

The concentrations of these sugars in potato (*Solanum tuberosum*) tubers and the grain of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rye (*Secale cereale*), rice (*Oryza sativa*) and maize (*Zea mays*) are given in Table 1. Sucrose, a disaccharide comprising glucose and fructose units (Fig. 1), is by far the most abundant sugar in tubers and grains, as it is in almost all plant tissues (this characteristic is unique to plants: other organisms do not synthesise or accumulate sucrose). Sucrose is also the major transported sugar in plants, a role to which it is particularly suited because sucrose solutions have relatively low viscosity, enabling high translocation rates. It is, of course, also a commodity in itself, sourced almost entirely from sugar cane and sugar beet.

Table 1 Concentration ranges (mmol kg^{-1}) of the major sugars in mature potato tubers (Amrein *et al*., 2003) and cereal grain: wheat (Muttucumaru *et al*., 2006), rye (Curtis *et al*., 2010), maize (Harrigan *et al*., 2007) and rice (Smyth *et al*., 1986)[a]

	Glucose	Fructose	Maltose	Sucrose
Wheat (*Triticum aestivum*)	1.49–4.84	0.80–1.89	2.81–6.40	21.90–25.65
Rye (*Secale cereale*)	0.64–33.43	0.61–7.02	0.74–21.05	26.81–49.52
Maize (*Zea mays*)	0.66–6.92	0.56–3.46	ND	12.91–89.60
Rice (*Oryza sativa*)	6.60–14.90 (total reducing sugars)			15.10–58.60
Potato (*Solanum tuberosum*)	0.5–14.2	0.2–4.5	ND	2.4–8.9

ND, not determined.

[a]All concentrations are given on a fresh weight basis. Sugar concentrations are affected by environmental as well as genetic factors and the figures should be considered as guidance values.

Glucose is the most abundant monosaccharide in tubers and grains, but not necessarily the most abundant

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reducing sugar. In wheat grain, for example, maltose is several times more abundant than glucose in varieties Solstice, Claire and Malacca, although glucose is more abundant in variety Hereward (Muttucumaru *et al.*, 2006). The difference arises because Hereward has an unusually high concentration of glucose (4.84 mmol kg^{-1} compared with 1.49–1.91 mmol kg^{-1} for the other varieties).

The other striking aspect of this table is the large range of concentrations that may be present. The concentration of sugars is affected by genotypic and environmental factors, and the values given in the table should therefore only be considered as a guide.

Sucrose synthesis

The pathway for sucrose synthesis is shown in Fig. 2. While almost all plant tissues have the capability of synthesising sucrose, most net synthesis occurs in photosynthetic leaves. The triose phosphate, dihydroxyacteone phosphate (DHAP), produced in the Calvin cycle, is exported from the chloroplasts and equilibrates with another triose phosphate, glyceraldehyde 3-phosphate (GAP) as a result of the action of triose phosphate isomerase. Dihydroxyacetone phosphate and GAP combine in an aldol condensation to produce fructose 1,6-bisphosphate. This is followed by the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate (F 6-P), a reaction catalysed by cytosolic fructose 1,6-bisphosphatase (FBPase). Fructose 6-phosphate can be reversibly converted to glucose 6-phosphate (G 6-P), the interconversion being catalysed by G 6-P isomerase (also known as phosphoglucose isomerase, or PGI, a dimeric enzyme with plastidic and cytosolic isoforms) (Thomas *et al.*, 1992; Nowitzki *et al.*, 1998). Glucose 6-phosphate also interconverts with glucose 1-phosphate (G 1-P), facilitated by phosphoglucomutase (PGM), another enzyme with plastidic and cytosolic forms. Cytosolic PGM is reviewed in depth by Periappuram *et al.* (2000).

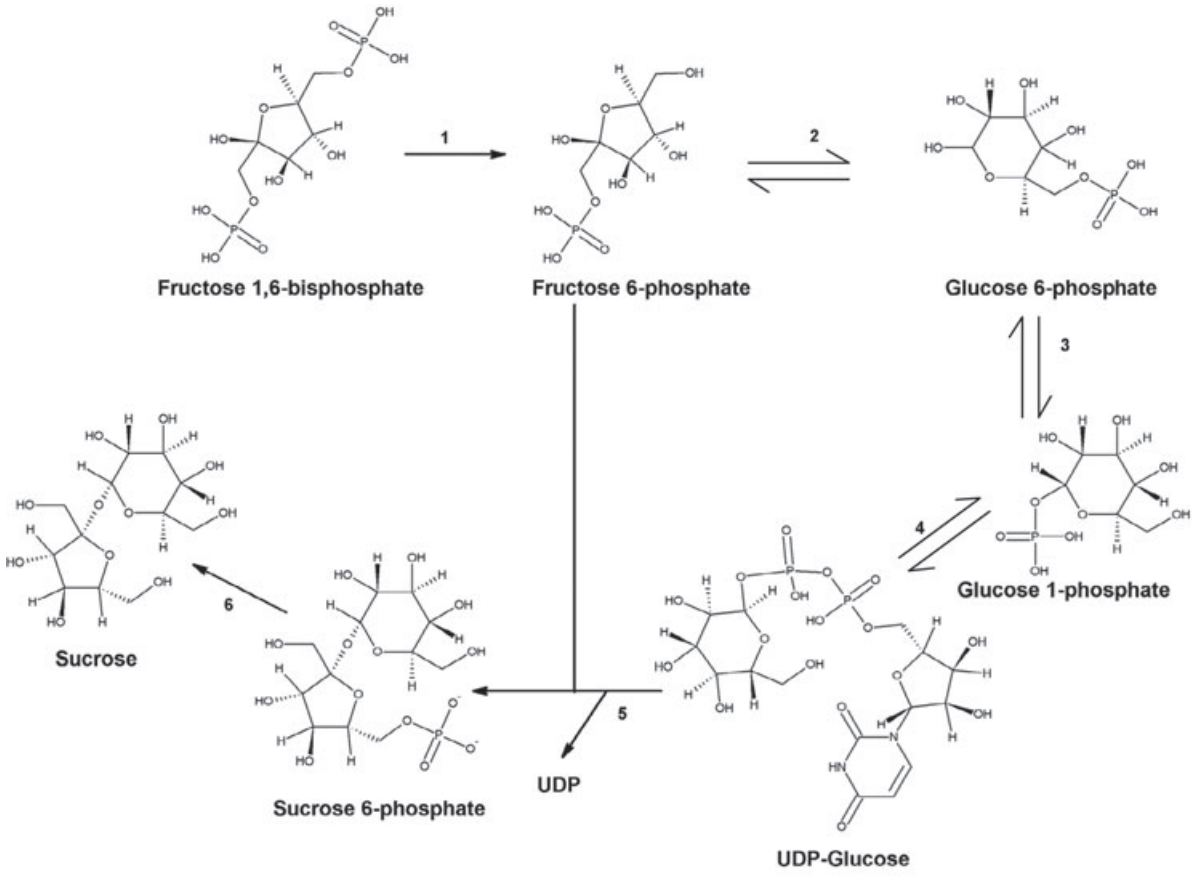

Figure 2 Pathway for sucrose synthesis from fructose 1,6-bisphosphate. The enzymes involved in the pathway are numbered: 1, cytosolic fructose 1,6-bisphosphatase; 2, glucose 6-phosphate isomerase (phosphoglucose isomerase); 3, phosphoglucomutase; 4, uridine diphosphate-glucose pyrophosphorylase; 5, sucrose phosphate synthase; 6, sucrose phosphate phosphatase.

Glucose 1-phosphate reacts with uridine triphosphate to produce uridine diphosphate (UDP)-glucose and pyrophosphate, a reversible reaction catalysed by UDP-glucose pyrophosphorylase. Uridine diphosphate-glucose pyrophosphorylase is encoded by two genes in *Arabidopsis* (Kleczkowski *et al*., 2004), but apparently only a single gene in barley and potato (Eimert *et al*., 1996; Sowokinos *et al*., 1997). Uridine diphosphate-glucose and F 6-P are used by sucrose phosphate synthase (SPS) to make sucrose phosphate; in effect the glucose unit from UDP-glucose being transferred to the F 6-P molecule. The phosphate group is removed from sucrose phosphate by sucrose phosphate phosphatase (SPP) to make sucrose.

The regulation of sucrose synthesis is reviewed in detail by Lunn & MacRae (2003). Sucrose phosphate synthase is encoded by a small multigene family comprising at least four members in *Arabidopsis* (Langenkämper *et al*., 2002) and SPS activity is regulated transcriptionally and post-transcriptionally through development and in response to environmental factors such as light and osmotic stress. The enzyme is activated allosterically by G 6-P and inhibited by inorganic phosphate (Pi) and is also regulated by phosphorylation (Sugden *et al*., 1999; Huang & Huber, 2001). Sucrose phosphate phosphatase is also encoded by a small multigene family, with four members in *Arabidopsis* and three in rice (Lunn & Furbank, 1999; Lunn, 2003).

The inorganic phosphate molecules released in the process of sucrose synthesis are returned to the chloroplast and used in the synthesis of more triose phosphate (Stitt & Heldt, 1985). Sucrose synthesis therefore has to be co-ordinated with photosynthesis, which is inhibited if too much sucrose accumulates (Stitt *et al*., 1988; Smith & Stitt, 2007).

Sucrose is transported from photosynthetic tissues to carbon sinks, including tubers and seeds, through the phloem sieve elements. Phloem loading at the source and unloading at the sink may occur symplastically through plasmodesmata linking mesophyll cells with the sieve elements, or apoplastically, involving export to the apoplast and transfer across the plasma membrane, a process mediated by proton-coupled sucrose transporters (SUT) (Lalonde *et al*., 1999, 2003). Different SUT transporters have different affinities for sucrose, SUT1 being a high affinity transporter and SUT4 a low affinity transporter, while SUT2 is a transporter that has been suggested to have a sensing function (Barker *et al*., 2000; Weise *et al*., 2000). All three proteins have been shown to be localised in sieve elements (Reinders *et al*., 2002).

Intriguingly, these SUT proteins are structurally related to hexose transporters of fungi and mammals. Budding yeast (*Saccharomyces cerevisiae*) has 17 hexose transporters encoded by *HXT* genes that are expressed differentially depending on the concentration of glucose that is available; high affinity, low capacity transporters are expressed when the glucose concentration is low, whereas low affinity, high capacity transporters are expressed when the glucose concentration is high (Kruckeberg, 1996; Boles & Hollenberg, 1997; Reifenberger *et al*., 1997). Budding yeast also has glucose sensors, SNF3 and RGT2, which are related to the transporters. The mammalian homologues are the GLUT transporters and a combined sensor/transporter, GLUT2 (Thorens, 2001).

There are two major crops for which sucrose is the main product, sugar beet and sugar cane; these provide 35 and 135 million tonnes, respectively, of sucrose per year, representing approximately 25% and 75% of global sucrose production. Sugar cane (*Saccharum* spp.) is a tropical monocotyledonous plant with C_4 photosynthesis, from which sucrose is extracted from stem tissues. In contrast, sugar beet (*Beta vulgaris*) is a temperate dicotyledonous species that is almost unique in that it accumulates sucrose in its storage root in preference to polysaccharides or oils.

Sucrose is used in the food sector, not only as a sweetener but also to thicken and provide texture. It is also used as a raw material for the production of high grade biodegradable plastics (especially for medical use), explosives, cosmetics, toiletries, detergents, agrochemical formulations and inks. It even finds uses in the construction industry, and in the textile industry for finishing fabrics. It is also used in ethanol production for biofuel. This industry has been established in Brazil for several decades but looks likely to spread more widely as demand for biofuel increases, although at current prices food uses of sucrose are more profitable than fuel uses, at least in developed countries.

Sucrose cleavage and primary carbon metabolism in storage organs

The fate of sucrose arriving at major sink organs such as seeds and tubers is a key determinant of crop quality and some of the pathways involved are shown in Fig. 3. In some plant tissues, incoming sucrose is hydrolysed to glucose and fructose by cell wall invertases and it is glucose and fructose that are imported into cells. Glucose is taken up by monosaccharide symporters called sugar transport proteins (STPs). These transporters are not involved in sucrose uptake or unloading and they do not transport fructose either; the route for fructose import still remains unclear. *Arabidopsis* has 6 cell wall invertases and 14 STP genes (Sherson *et al*., 2003), one of which, *AtSTP1*, encodes a protein that transports galactose, xylose and mannose as well as glucose, and responds sensitively to extracellular sugar availability.

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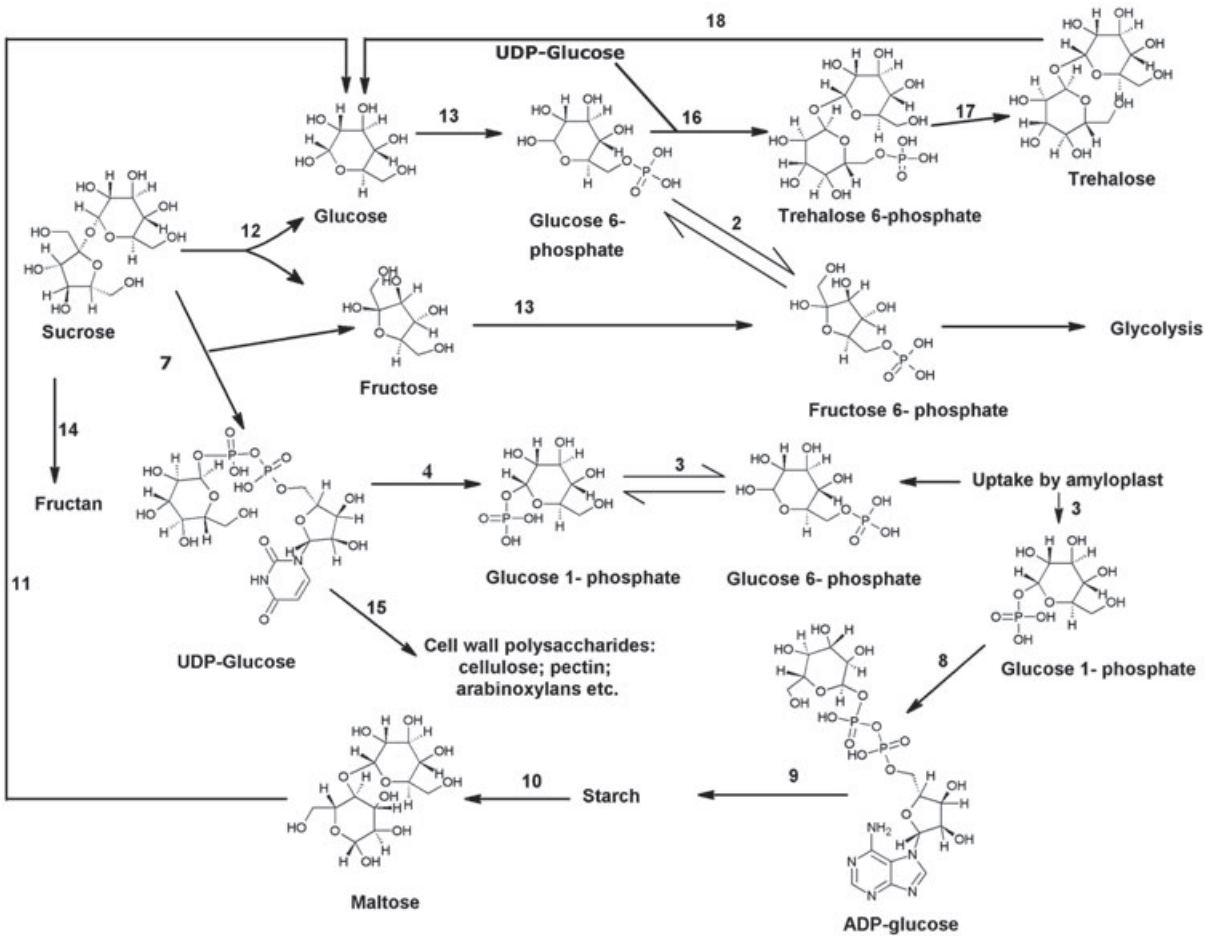

Figure 3 Pathways for sucrose breakdown and utilisation. The enzymes involved in the pathways are numbered, overlapping and following on from the numbering given in Fig. 2: 2, glucose 6-phosphate isomerase (phosphoglucose isomerase); 3, phosphoglucomutase; 4, uridine diphosphate-glucose pyrophosphorylase; 7, sucrose synthase; 8, adenosine diphosphate-glucose pyrophosphorylase (note that this is plastidic in most plant species and tissues, but cereal endosperms express a cytosolic form of the enzyme); 9, suite of starch synthesis enzymes (see text); 10, suite of starch-degrading enzymes (see text); 11, maltase; 12, invertase; 13, hexokinase; 14, fructosyl transferases; 15, cellulose synthase and other enzymes for cell wall polysaccharide synthesis (see text); 16, trehalose phosphate synthase; 17, trehalose phosphate phosphatase; 18, trehalase.

Invertases (β-fructofuranosidase) are also present in the apoplast, vacuole, cytosol, plastids and mitochondria. The hydrolysis of sucrose by invertases is irreversible and the name invertase derives from the mixture of glucose and fructose produced by the reaction, which in the food industry is referred to as inverted sugar syrup. Plant invertases can be classified according to their pH optima, as well as their location: acid invertases are present in the cell wall, apoplast and vacuole, and neutral/alkaline invertases in the cytosol and plastids. Acid and neutral/alkaline invertases are structurally unrelated. In addition to their role in phloem unloading in some species, invertases are involved in supplying hexoses for glycolysis, respiration and secondary product formation, responding to wounding and pathogen attack, osmoregulation and the response to cold stress (reviewed by Roitsch & González, 2004). Neutral/alkaline invertases are found only in plants and photosynthetic bacteria (Vargas & Salerno, 2010).

The glucose and fructose produced by invertase action on sucrose are substrates for hexokinases (Fig. 3), which catalyse the phosphorylation of hexoses to produce hexose phosphates. The primary function of hexokinases is to supply hexose phosphates for glycolysis. In plants they are encoded by small multigene families: rice, for example, has nine family members (Cho *et al*., 2006) and *Arabidopsis* six (Rolland *et al*., 2002; Claeyssen & Rivoal, 2007). However, it appears that not all are catalytically active (Karve *et al*., 2008) and some have signalling as well as catalytic activity (Moore *et al*., 2003), as we discuss later.

The G 6-P that is produced from glucose is converted to F 6-P by G 6-P isomerase, an enzyme that we discussed in the context of sucrose synthesis, and the F 6-P enters glycolysis (Fig. 3).

Plants possess a second enzyme that cleaves sucrose: sucrose synthase (SuSy). Sucrose synthase is present in the cell wall and cytoplasm and catalyses the reversible conversion of sucrose and UDP to UDP-glucose and fructose. Despite the name of the enzyme, the equilibrium of the reaction under physiological conditions very much favours cleavage over synthesis, particularly in storage and vascular tissues where sucrose concentrations are high. Sucrose synthase activity increases in response to sucrose but is not affected by glucose (Salanoubat & Belliard, 1989; Sowokinos & Varns, 1992). *SuSy* gene expression may also be induced by sucrose: potato, for example, has sucrose-inducible and sucrose-uninducible *SuSy* genes (Fu & Park, 1995). *Arabidopsis* contains six *SuSy* genes, with different spatial and temporal expression patterns (Bieniawska *et al*., 2007).

The production of UDP-glucose is the first step in the predominant route for synthesis of many polysaccharides, including starch for storage and cell wall polysaccharides such as cellulose, pectin and arabinoxylans, and this, rather than fuelling glycolysis, is the primary function of SuSy under normal circumstances. Indeed, SuSy activity is closely correlated with starch accumulation in, for example, maize grain and potato tubers (Chourey & Nelson, 1976; Zrenner *et al*., 1995), while over-expression of invertase and hexokinase in potato causes a dramatic reduction in starch synthesis and over-accumulation of glycolytic intermediates (Trethewey *et al*., 1998). These data suggest that SuSy and invertase activities are not interchangeable. Indeed, the relative activity of SuSy and invertase may be a key determinant of carbon partitioning and has been proposed to play a role in the control of development, with an increase in sucrose synthase and decrease in invertase activity triggering the initiation of the storage phase of, for example, legume seed development (Weber *et al*., 1996). However, if the SuSy and invertase pathways are not interchangeable, they must be compartmentalised in some way, because they both involve the production of G 6-P, and as yet there is no known mechanism for that to occur. Furthermore, SuSy has been shown to sustain glycolysis in tomato and *Arabidopsis* roots under hypoxic conditions (Germain *et al*., 1997; Bieniawska *et al*., 2007), while mutant *Arabidopsis* plants lacking SuSy activity have been shown to grow normally and synthesise starch and cell wall polysaccharides (Barratt *et al*., 2009).

Storage polysaccharides: starch and fructan

Starch biosynthesis and uses

Starch is a complex polysaccharide comprising linear chains of glucose units joined by α-1,4-glycosidic bonds (amylose) and branched molecules consisting of short linear chains linked at branch-points by α-1,6-glycosidic bonds (amylopectin). The first step in the predominant route for its biosynthesis is the conversion of UDP-glucose to G 1-P (Fig. 3), the reversible conversion that is catalysed by UDP-glucose pyrophosphorylase. Glucose 1-phosphate is the molecule that together with adenosine triphosphate (ATP) is used by adenosine diphosphate (ADP)-glucose pyrophosphorylase to make ADP-glucose and pyrophosphate. However, in most plant tissues this reaction is plastidic, and it appears that G 6-P is the predominant glucose phosphate taken up by amyloplasts, rather than G 1-P. So, G 1-P is first converted to G 6-P by cytosolic PGM, and the G 6-P is converted back to G 1-P by plastidic PGM. Cereal endosperm, on the other hand, expresses a cytosolic ADP-glucose pyrophosphorylase (Beckles *et al*., 2001) and ADP-glucose is taken up by the plastids via a specific transporter (Shannon *et al*., 1998).

Adenosine diphosphate-glucose pyrophosphorylase is a heterotetrameric enzyme in plants, comprising two large and two small subunits. Mutations in the genes encoding either subunit have profound effects on starch synthesis; in maize, for example, the large subunit is encoded by the gene *Shrunken2* (*Sh2*) (Hannah *et al*., 1976) and the small subunit by *Brittle2* (*Bt2*) (Bae *et al*., 1990), the gene names deriving from the grain phenotype caused by loss of function mutations. Adenosine diphosphate-glucose pyrophosphorylase activity is also regarded as an important determinant of sink strength in cereals, and increased activity of the enzyme in wheat has been shown to result in an increase in grain yield (Smidansky *et al*., 2002).

Adenosine diphosphate-glucose is the glucose donor for starch biosynthesis (Fig. 3). Amylose is synthesised by starch synthases that are bound to starch granules, called granule-bound starch synthases (GBSS), while amylopectin is synthesised by soluble starch synthases in conjunction with starch branching enzyme. Starch synthesis in *Arabidopsis* was reviewed by Zeeman *et al*. (2002).

Starch from cereals, potato, sweet potato and cassava is the most important carbohydrate in the human and farmed animal diet. Over 2 billion tonnes of starch are produced annually in cereal grain and 700 million tonnes in roots and tubers (Tester & Karkalas, 2002). In developed countries, starch provides 35% of daily calorific intake, while in developing countries it may provide 80% (Burrell, 2003). Starch is also used as a thickener

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in the food industry and to produce sugars through enzymatic digestion. Until recently these sugars were used largely in the food industry, but over the last few years there has been a huge increase in the use of sugars derived from starch for the production of ethanol for fuel. This industry is now well-established in the USA, where the annual growth rate in ethanol production, almost entirely from maize starch, was 25% between 2003 and 2007 (Dhuyvetter *et al*., 2008) and in 2009–2010 is predicted to take a third of the US maize crop. Other industrial uses include papermaking (starch typically makes up 8% of a sheet of paper), adhesive production, gypsum wall board manufacture and strengthening textile yarns.

A difficulty in using starch for industrial purposes or as a food additive is that the two components, amylose and amylopectin, have different characteristics and have to be separated or modified chemically before use. Amylose, for example, has gelling properties that are undesirable in some processes. A maize mutant with starch containing almost no amylose was discovered in China in the early 20th Century (Collins, 1909). This and similar mutants were given the name waxy maize, and the gene carrying the mutation was called *waxy* or *wx*; it was subsequently found to encode a GBSS (Shure *et al*., 1983). Waxy maize has been grown to produce starch for industrial purposes since the 1940s. Much more recently, BASF have developed a genetically modified potato, marketed as 'Amflora', in which the activity of GBSS is greatly reduced. The starch from this new potato variety is composed almost entirely of amylopectin, whereas normal potato starch contains approximately 20% amylose. Amflora was developed for the European market and was mired in the European Union's tortuous regulations covering the use of genetically modified crops for over a decade. It was finally approved for cultivation in 2010.

The demand for starch and starch-containing crops has been growing more rapidly than starch production for many years, culminating in stocks reaching alarmingly low levels in 2005–2006 and a consequent steep rise in prices. This led to a controversy over the use of starch crops for non-food purposes, particularly ethanol for biofuel. As they generally do, farmers responded to the increase in prices by increasing production, and prices fell back again. However, the long-term trend in demand continues upwards and meeting that demand in the coming years will be a challenge.

Starch degradation, maltose and malting

Starch breakdown in cereal grain has been the subject of intense study because of the key role that it plays in germination and malting. Stored starch is broken down to provide the sugars required for growth and development. This process is initiated by the hormone gibberellic acid, which is released by the embryo and aleurone following the detection of environmental cues indicating that conditions are suitable for germination (note that by seed maturity the endosperm is desiccated and dead). When germination is initiated, α-amylase is synthesised by the aleurone; this is an endoamylase that acts at random locations along the starch chain to yield shorter glucan chains, facilitating the action of other degrading enzymes. Ultimately its products are maltotriose, maltose and limit dextrin (a mixture of branched and unbranched glucans). Proteases are also synthesised and the enzyme β-amylase, which accumulates during seed development, is converted from its inactive, bound form, in which it is linked to insoluble proteins by disulphide bridges, to its soluble, active form. β-Amylase attacks the non-reducing end of a glucan chain, catalysing the hydrolysis of alternate α-1,4 glycosidic bonds, cleaving off a maltose molecule each time. Starch breakdown also involves debranching enzyme (limit dextrinase). The joint enzymatic capability of these enzymes is known as the grain diastatic power and is an important target trait for breeding cereal varieties for malting (Ziegler, 1999). The maltose produced as a result of starch degradation may be converted to hexoses or transported to the embryo via SUT symporters in the scutellum (Aoki *et al*., 2006).

For many years, starch degradation was assumed to proceed in this way in all plant species and tissues, but recent evidence has shown that this is not the case. Starch phosphorylase (α-1,4 glucan phosphorylase), for example, which acts on the terminal α-1,4-glycosidic bond to release G 1-P, appears to be particularly important in potato tubers (Malone *et al*., 2006; Rommens *et al*., 2006; Morales *et al*., 2008). In *Arabidopsis* leaves, the synthesis and degradation of transitory starch is under diurnal regulation, with starch accumulating during the day and being mobilised during the night (reviewed by Zeeman *et al*., 2007). Starch degradation first requires the phosphorylation of amylopectin by α-glucan water dikinase (GWD) and phosphoglucan water dikinase (PWD) (as its name suggests, PWD acts on amylopectin that is already phosphorylated, so PWD is dependent on GWD). *Arabidopsis* leaves contain at least three enzymes that can then degrade the starch: these are α-amylase, β-amylase and debranching enzyme. Unexpectedly, mutants lacking α-amylase activity have been shown to break starch down normally (Yu *et al*., 2005), suggesting that α-amylase does not play a crucial role in starch breakdown in *Arabidopsis* leaves. Starch degradation is reviewed in detail by Smith *et al*. (2005).

The detection of diurnal fluctuations in cytosolic maltose concentration in leaves of *Arabidopsis* led to

the identification of maltose as the major product of the catabolism of transitory starch in chloroplasts (Weise *et al*., 2004). The maltose that is produced is transported to the cytosol by the membrane transporter, MEX1, and sucrose is synthesised for transport to respiring tissues (Niittylä *et al*., 2004).

The normal diurnal peaks of maltose accumulation in the cytosol can be disrupted if *Arabidopsis* is exposed to temperature shock: both freezing and heat shock have been shown to induce the up-regulation of β-amylase, resulting in the accumulation of maltose (Kaplan & Guy, 2004). Maltose has been proposed to confer protection against degradation of PSII and the suppression of maltose production by RNA interference of β-amylase results in decreased PSII photochemical efficiency (Kaplan & Guy, 2005). Developing barley grain has also been found to have increased β-amylase activity under stress. This affects grain filling and is mediated by interactions between β-amylase, abscisic acid (ABA) and hydrogen peroxide (Wei *et al*., 2009).

The production of maltose from cereal grain starch reserves is important in the brewing and distilling industries. During malting, grains of barley, rye or wheat are exposed to moist, warm conditions that induce partial germination and allow starch-degrading enzymes to be synthesised. Germination is halted before the starch is broken down by drying the partially germinated grains in a kiln. The grains are then roasted at high temperatures, allowing the development of colour and flavour via the Maillard reaction (Pollock, 1962), which is described below. They are then crushed and milled into a grist, added to hot water and subjected to a series of controlled, high-temperature (<100°C) 'stands' in a process known as mashing. Ideally, it is during mashing that most starch hydrolysis occurs, producing the sugars required for fermentation.

Increased efficiency of the enzymatic degradation of starch to fermentable sugars is a possible method of improving the yield and economic viability of this process. High-temperature mashing improves the reaction kinetics, but protein denaturation becomes a problem (for example, β-amylase loses its conformation) and sugar production is reduced. Incorporating thermostable enzymes into the process is therefore an attractive prospect and there is interest from biotechnologists in using enzymes from thermophilic bacteria that retain their structure and function at high temperatures. Lin *et al*. (2008) showed that there is potential for this approach by transforming potato to express a β-amylase gene from the bacterium *Clostridium thermosulfurogenes*. The tubers of the transgenic lines had higher β-amylase activity at 60°C than the wild type. The results were not conclusive because other aspects of tuber chemistry were altered in the transgenic plants. However, the study did suggest promise for this technique for increasing the efficiency of malting technologies. In another study, barley was transformed with a mutant β-amylase gene encoding an enzyme with an optimal temperature 11.6°C higher than the wild type, resulting in an increase in the diastatic power (Kihara *et al*., 2000).

Low amylase activity and inefficient starch degradation result in the production of beer with a hazy appearance and low alcohol content. This is a particular problem in the production of beer from sorghum, which is a common practice in Africa (Ogbonna, 1992). On the other hand, too much amylase activity before harvest is detrimental to grain quality, lowering the Hagberg falling number and hence the value of the grain. Hagberg falling number is an international standard determined by the time it takes for a steel ball to fall through a slurry of flour and water that has been heated to release the starch and partially gelatinise it. If starch has been partly hydrolysed by α-amylase produced in the developing grain, the slurry is less viscous and the ball falls more quickly. The usual cause of premature high amylase activity is preharvest sprouting (Masojć & Milczarski, 2009), but α-amylase activity in the endosperm can also be induced by a sudden decrease in temperature during the middle to late stages of grain ripening (Gooding *et al*., 2003; Mares & Mrva, 2008). This is known as late maturity α-amylase or prematurity α-amylase. α-Amylase may also be retained in the pericarp (retained pericarp α-amylase); this usually vanishes with grain desiccation but in humid weather conditions may persist to grain maturity and harvest (Kettlewell *et al*., 1996).

Fructan

The other major storage polysaccharide made by plants is fructan. Indeed, fructan is the major reserve carbohydrate in approximately 15% of higher plants and accumulates in the vegetative tissues of many species, including temperate forage grasses and cereals such as wheat, barley and rye (Cairns *et al*., 2000). Fructan consists of linear and branched polymers of fructose with a glucose unit at the head of the chain. Typically, it is found in species that are adapted to temperate climates with regular periods of drought and/or frost. Fructan is produced directly from sucrose and is synthesised and accumulates in the vacuole. A family of enzymes is involved, collectively known as fructosyl transferases. These enzymes transfer fructose from sucrose to fructan chains and are related to acid invertases. They are reviewed in detail by Vijn & Smeekens (1999) and Ritsema & Smeekens (2003). Fructan is broken down by fructan exohydrolases, which

sequentially release fructose monomers from the end of the fructan chain (Van den Ende *et al.*, 2004).

There are five classes of plant fructan, based on chain length, branching and fructosyl linkages: these are called inulin, levan, mixed levan, inulin neoseries and levan neoseries. The simplest of these is inulin, which is a linear chain of (2-1)-linked β-D-fructosyl units, and the simplest inulin is the trisaccharide, 1-kestose, which comprises two fructose units and one glucose unit. Wheat and barley fructan is mostly mixed levan, which is composed of both (2-1)- and (2-6)-linked β-D-fructosyl units (Carpita *et al.*, 1989; Bonnett *et al.*, 1997).

The fact that fructan is present for the most part in temperate plants that have to survive periods of cold and/or drought has led to the hypothesis that it plays a role in osmotic regulation in response to these stresses (Wiemken *et al.*, 1995). Consistent with this hypothesis, fructan has been shown to accumulate in response to drought and cold stress, as well as nitrogen deficiency (Livingston, 1991; Wang & Tillberg, 1996; Volaire & Lelièvre, 1997; Kerepesi *et al.*, 1998) and transgenic tobacco plants engineered to synthesise fructan (tobacco does not normally make fructan) have been shown to have improved osmotic stress tolerance (Pilon-Smits *et al.*, 1995).

Cell wall polysaccharides

As we have described, the first step in the predominant route for starch biosynthesis is the conversion of UDP-glucose to G 1-P (Fig. 3). Uridine diphosphate-glucose is also used in the formation of cell wall polysaccharides. These include cellulose, a glucan comprising linear chains of glucose molecules joined by β-1,4 glycosyl linkages, (1,3;1,4)-β-glucan, which is similar to cellulose but has one β (1,3) linkage for every three or four β (1,4) linkages, xyloglucans and xylans. Xyloglucan has a backbone of β (1,4)-linked glucose residues, some of which are substituted with (1,6)-linked xylose side-chains. It is a major cell wall polymer of dicotyledonous plants. Xylans are polymers of the pentose, xylose, for which UDP-xylose is the xylose donor; UDP-xylose is produced from UDP-glucose by dehydrogenation to UDP-glucuronate followed by decarboxylation. The major cell wall polymers of grasses are arabinoxylans, which are xylans in which some of the xylosyl units are subsituted with another pentose, arabinose, a stereoisomer of xylose. A proportion of the arabinose units are esterified with ferulic acid to form arabinofuranosyl side-chains.

The other major component of plant cell walls is pectin, which is comprised of another complex group of polysaccharides. These include homogalacturonans, which are linear chains of α-(1-4)-linked galacturonic acid (an oxidised form of galactose), substituted galacturonans, which contain side-chains of xylose or apiose (another pentose) on a galacturonic acid backbone, and rhamnogalacturonans, which contain a backbone with repeating units of galacturonic acid and rhamnose (rhamnose is a deoxyhexose), with various sugar side-chains. Pectin breakdown by pectinase and pectinesterase is an important part of fruit ripening and leaf abscission.

These cell wall polymers are important because they make up a significant proportion of plant biomass and are the main contributers to soluble fibre in the diet. Increasing the levels of β-glucan in barley grain, for example, is claimed to reduce its glycaemic index, and barley varieties have therefore been bred to contain high levels of β-glucan (for example, BARLEYmax™, developed by CSIRO, Victoria, Australia), aimed at the breakfast cereal market. Confusingly, such varieties are often described as having 'resistant starch', although the β-glucan is derived from cell walls, not starch. These varieties are not suitable for beer-making because the β-glucan causes haziness. A detailed examination of the structure and synthesis of cell wall polysaccharides is beyond the scope of this review but has been provided elsewhere (Doblin *et al.*, 2002; Somerville *et al.*, 2004; Somerville, 2006; Brown *et al.*, 2007; Mitchell *et al.*, 2007).

Sugars as signalling molecules

In 1990, Jen Sheen at the University of Harvard showed that the promoters of seven maize photosynthetic genes were repressed by glucose or sucrose in a maize protoplast system (Sheen, 1990). Genes encoding enzymes of the glyoxylate cycle, carbohydrate metabolism, defence responses and storage proteins have since been shown to respond to sugars (reviewed by Halford & Paul, 2003) and, in a transcriptomic study, Price *et al.* (2004) showed that almost a thousand *Arabidopsis* genes were up- or down-regulated by glucose. These included genes involved in biotic and abiotic stress responses, carbohydrate metabolism, nitrogen metabolism, lipid metabolism, inositol metabolism, secondary metabolism, nucleic acid-related activities, protein synthesis and degradation, transport, signal transduction, hormone synthesis and cell growth or structure.

These studies showed that plants are able to sense and respond to sugar levels and, indeed, sugars can be regarded as hormone-like signalling molecules. There has been considerable progress in elucidating the signalling networks that are involved and this has revealed remarkable conservation of ancient signalling systems shared by fungi, animals and plants. For example, the overriding mechanism controlling carbon metabolism in budding yeast is glucose repression. Glucose affects the

synthesis of dozens of enzymes in this organism, the utilisation of alternative carbon sources, gluconeogenesis, respiration and the biogenesis of mitochondria and peroxisomes (Dickinson, 1999). Central to glucose repression signalling in budding yeast is a protein kinase called sucrose nonfermenting-1 (SNF1) (Celenza & Carlson, 1986). Remarkably, homologues of SNF1 are present in animals [AMP-activated protein kinase (AMPK)] and plants [SNF1-related protein kinase-1 (SnRK1)].

These protein kinases are reviewed in depth elsewhere (Halford & Hey, 2009; Hey *et al.*, 2010) and we will not describe them in detail here, but it is important to summarise what is known about SnRK1 because it affects many of the processes described above. Firstly, there is considerable evidence that SnRK1 is required for starch biosynthesis. It has been shown to be required for sucrose synthase and ADP-glucose pyrophosphorylase gene expression in potato, for example (Purcell *et al.*, 1998; McKibbin *et al.*, 2006), and its over-expression causes an increase in starch content and a decrease in the concentration of glucose in potato tubers (McKibbin *et al.*, 2006). It is also required for the sucrose-dependent redox activation of ADP-glucose pyrophosphorylase (Tiessen *et al.*, 2003), so it potentially regulates starch synthesis through two enzymes and through both transcriptional and post-transcriptional mechanisms. Evidence of a role for SnRK1 in regulating starch synthesis has also been obtained in rice (Kanegae *et al.*, 2005) and sorghum (Jain *et al.*, 2008). Similarly, SNF1 and AMPK are required for the synthesis of glycogen, the major storage polysaccharide of fungi and mammals, although the mechanisms through which they regulate the process are different (the glucose donor for glycogen synthesis is UDP-glucose, not ADP-glucose, and fungi and animals lack both sucrose synthase and ADP-glucose pyrophosphorylase).

Paradoxically, SnRK1 may also be involved in regulating starch breakdown because it has been implicated in the regulation of α-amylase gene expression in both wheat and rice (Laurie *et al.*, 2003; Lu *et al.*, 2007) and with starch mobilisation in response to the onset of darkness in *Physcomitrella* (Thelander *et al.*, 2004). In contrast to sucrose synthase and ADP-glucose pyrophosphorylase genes, which are sucrose inducible, α-amylase genes are glucose repressible. SnRK1 has also been shown to regulate a sugar-repressed asparagine synthetase gene in *Arabidopsis* (Baena-González *et al.*, 2007), linking sugar signalling with amino acid metabolism. The influence of SnRK1 goes much wider even than that, and it has been suggested that SnRK1 and related protein kinases provide an interface between metabolic and stress signalling (Baena-González *et al.*, 2007; Halford & Hey, 2009; Hey *et al.*, 2010). The paradox of SnRK1 being involved in regulating both starch synthesis and breakdown is reviewed in more detail by Halford & Hey (2009).

Similar to its animal and fungal counterparts, SnRK1 regulates enzyme activity directly as well as acting through the regulation of gene expression. The enzymes known to be phosphorylated by SnRK1 include one of those shown in Fig. 2, SPS (Sugden *et al.*, 1999), and TPS5, which is a member of the trehalose phosphate synthase (TPS) family but has not been demonstrated to have TPS activity (Harthill *et al.*, 2006). Another, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (F2KP) (Kulma *et al.*, 2004), controls the levels of the metabolite fructose 2,6-bisphosphate, which inhibits another enzyme that features in Fig. 2, FBPase (Stitt, 1990). The other enzymes that have been shown to be directly phosphorylated and inactivated by SnRK1 are nitrate reductase (NR), which is a key enzyme in the assimilation of inorganic nitrogen, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), which catalyses the NADPH-dependent reduction of HMG-CoA to mevalonic acid (Ball *et al.*, 1995; Douglas *et al.*, 1997). The latter is the first step in sterol synthesis and transformation of tobacco with genes encoding modified HMG-CoA reductases lacking an SnRK1 target site results in significant increases in the accumulation of seed sterols (Hey *et al.*, 2006). Note that in the case of NR, TPS and F2KP, inactivation of the enzyme also requires the binding of a 14-3-3 protein. The importance of these enzymes and the range of metabolic processes that they are involved in show just how wide the influence of SnRK1 and thereby sugar signalling is in plant cells.

Demonstrating a clear effect of a metabolite on SnRK1 activity is notoriously difficult. Feeding experiments are most easily performed on cultured plant material, but such material typically has high levels of SnRK1 activity no matter what it is fed (unpublished data). Distinguishing between the effects of sucrose and hexoses is made difficult by the fact that the levels of these metabolites within the cell may or may not be linked, depending on the presence or absence of enzymes such as invertase, sucrose synthase and SPS that interconvert them. For instance, one of the first examples of sugar-regulated gene expression to be demonstrated in plants was that of α-amylase in rice root cultures (Yu *et al.*, 1991; Huang *et al.*, 1993). Sucrose feeding was shown to inhibit expression of the α-amylase gene and, ever since, α-amylase gene expression has commonly been referred to as sucrose repressible. In fact, sucrose is cleaved extracellularly by a cell wall-associated invertase prior to uptake by cultured rice cells (Amino & Tazawa, 1988) and it is almost certainly intracellular glucose

rather than extracellular sucrose concentration that influences α-amylase gene expression in this system.

Two metabolites that have been shown to inhibit SnRK1 are G 6-P (Toroser *et al.*, 2000) and trehalose 6-phosphate (T 6-P) (Zhang *et al.*, 2009). Trehalose 6-phosphate is the more potent inhibitor of the two but it requires a hitherto unidentified intermediary factor to have its effect. Trehalose 6-phosphate is an intermediate in the trehalose biosynthetic pathway: trehalose is a non-reducing disaccharide comprising two glucose units joined by an α-1,1-glycosidic bond (Fig. 1). The biosynthetic pathway for this disaccharide was regarded for many years as an insignificant offshoot from the major flow of carbon metabolism because trehalose hardly accumulates at all in plants, with the exception of a few resurrection plants. Plants are unusual in this respect because trehalose is actually more widespread in nature than sucrose, and despite the fact that plants do not generally accumulate trehalose, they do have the capacity to synthesise and degrade it (Bianchi *et al.*, 1993; Drennan *et al.*, 1993; Goddijn & van Dun, 1999). Interest in the pathway was stimulated just over a decade ago by the discovery that the trehalose synthesis pathway plays an important role in metabolic signalling in plants (Goddijn & Smeekens, 1998; Eastmond *et al.*, 2002; Schluepmann *et al.*, 2003). The interaction between T 6-P and SnRK1 provides a mechanism through which this could occur.

The pathway involves two enzymes, TPS, which catalyses the formation of T 6-P from G 6-P and UDP-glucose, and trehalose phosphate phosphatase (TPP), which catalyses the dephosphorylation of T 6-P to trehalose. Trehalose is cleaved to form two glucose molecules by another enzyme, trehalase. In fungi, both the enzyme, TPS, and the metabolite, T 6-P, have an inhibitory effect on hexokinase, thereby regulating the flow of carbon into glycolysis and ensuring that cellular ATP levels are not depleted by 'overactivity' of hexokinase (Bonini *et al.*, 2000; Noubhani *et al.*, 2000). That does not appear to occur in plants, but T 6-P does play a role in regulating flow into glycolysis: transgenic and mutant plants with reduced T 6-P content cannot grow on sugar-containing media because they accumulate large pools of metabolites and ATP levels become depleted, while transgenic plants with elevated T 6-P levels grow better than wild type on sugars (Schluepmann *et al.*, 2003).

In addition to the role of the trehalose pathway in signalling, the presence of relatively high concentrations of trehalose in resurrection plants suggests that trehalose may act as an osmoprotectant and transgenic plants that have been engineered to accumulate it do show improvements in stress tolerance (Pilon-Smits *et al.*, 1999; Garg *et al.*, 2002).

Another factor that has been implicated in sugar sensing and signalling is the hexokinase family of enzymes (Jang *et al.*, 1997). As we have discussed above, hexokinases have a metabolic function in catalysing the conversion of hexoses to hexose phosphates (Fig. 3), but some may also have a role in sensing and signalling glucose levels. The notion that hexokinases might have such a function has been controversial (Halford *et al.*, 1999) but it is now widely accepted (Rolland *et al.*, 2001; Karve *et al.*, 2008).

The mechanism by which hexokinases sense glucose and initiate a signal is not known and hexokinase signalling activity therefore cannot be measured. However, in addition to gene expression, hexokinase signalling appears to affect cell proliferation, root and inflorescence growth, leaf expansion and senescence. Some of these effects may be mediated through cross-talk with light and hormone signalling (Wingler *et al.*, 1998; Hwang & Sheen, 2001). There is also evidence that hexokinase signalling interacts with auxin signalling to promote or inhibit growth (Moore *et al.*, 2003).

The Maillard reaction

Sugars have a sweet taste, of course, but they also have a major impact on the processing quality of plant-derived raw materials through their participation in the Maillard reaction. This reaction, which was named after the French chemist, Louis Camille Maillard, who first described it in 1912 (Maillard, 1912), comprises a series of non-enzymatic reactions between sugars and amino groups, principally those of amino acids. The Maillard reaction is promoted by high temperature and low moisture content and, therefore, occurs mainly in cooked foods prepared by frying, baking and roasting. The reaction requires a reducing sugar such as glucose, fructose or maltose. Sucrose does not participate unless it is hydrolysed through enzymatic, thermal or acid-catalysed reaction (De Vleeschouwer *et al.*, 2009). The products of the Maillard reaction include melanoidin pigments, which are complex polymers that are responsible for the brown colour in fried, baked and roasted foods. The reaction also provides complex mixtures of compounds that impart flavour and aroma. Heterocyclic compounds produced in the Maillard reaction that contribute to aroma include pyrazines, pyrroles, furans, oxazoles, thiazoles and thiophenes (Mottram, 2007). The particular compounds formed give different cooked foods their characteristic aroma and depend on the amino acid and sugar composition of the food and the processing conditions.

The steps in the Maillard reaction as they are understood today (Fig. 4) were first proposed by an American

chemist, John Hodge, in 1953 (Hodge, 1953) and are described in detail by Nursten (2005) and Mottram (2007). The reaction is initiated by the condensation of the carbonyl (C-O) group of a reducing sugar with the amino group of an amino acid or other amino compound, producing a Schiff base (Fig. 4). If the sugar is an aldose, the Schiff base cyclises to give an N-substituted aldosylamine, such as glucosylamine from glucose. Acid-catalysed rearrangement of the aldosylamine gives a 1,2-enaminol, which is in equilibrium with its ketotautomer, an N-substituted 1-amino-2-deoxyketose: these are known as Amadori rearrangement products. Ketoses, such as fructose, give related Heyns rearrangement products by similar pathways.

In the second stage of the reaction, the Amadori and Heyns rearrangement products undergo enolisation, deamination, dehydration and fragmentation, giving rise to sugar dehydration and fragmentation products containing one or more carbonyl groups, including heterocyclic furfurals, furanones and pyranones (Fig. 4). These carbonyl compounds produced in the reaction may contribute to flavour characteristics in their own right (Mottram, 2007), but carbonyls are reactive species that can undergo condensation reactions with amino groups and other components present at this stage of the Maillard reaction, resulting in the formation of many different flavour compounds. Fig. 5 summarises some of these reactions. An important reaction of carbonyl compounds is Strecker degradation, whereby they bring about the deamination and decarboxylation of an amino acid to give an aldehyde in which the α-carbon of the amino acid is converted to an aldehyde group; this reaction also yields an α-aminoketone (Fig. 5). Strecker degradation of cysteine is very important in flavour generation because it gives rise to hydrogen sulphide and ammonia which are important intermediates for the introduction of nitrogen and sulphur into heterocyclic flavour compounds, such as pyrazines, oxazoles and thiazoles (Fig. 5) (Mottram, 2007).

The Maillard reaction is very complex and more information can be found in reviews by Ledl & Schleicher (1990), Nursten (2005) and Mottram (2007), among others. While many of its products contribute to the flavour, colour and aroma of food, some are undesirable (Friedman, 2005). Acrylamide, for example, which was shown to be present in mainly plant-derived foods after high-temperature cooking and processing (Tareke *et al*., 2002) is a product of the Maillard reaction (Mottram *et al*., 2002; Stadler *et al*., 2002). The major route for its formation is a Strecker-type reaction involving sugar-derived carbonyl compounds and asparagine (Zyzak *et al*., 2003). Acrylamide is neurotoxic, carcinogenic and genotoxic in rodents and has been classified as a probable human carcinogen by the World Health Organisation (Friedman, 2003). The reduction of acrylamide levels in food is now recognised as a difficult and important problem for the agricultural and food industries. Baked, roasted and fried potato and cereal foods make a significant contribution to dietary intake of acrylamide, which for adults is estimated to be approximately 0.3–0.6 μg per kg body weight per day, with the intake for teenagers and children being even higher on a per bodyweight basis (Mucci & Wilson, 2008). Other potentially harmful compounds derived from the Maillard reaction include heterocyclic aromatic amines in grilled meat (Skog *et al*., 1998). Recently it has been suggested that more Maillard products could be toxic and some attention has been directed towards furan (Leopardi *et al*., 2010).

Factors affecting sugar levels in crops

For the two crops that supply us with sucrose, sugar cane and sugar beet, increased sucrose yield would obviously be desirable, but there has been surprisingly little work on the genetic, environmental and agronomic/management factors that affect sucrose accumulation in either species. In sugar beet, sucrose makes up over 75% of the root dry matter or approximately 17.5% of the fresh weight. The sucrose concentration was doubled in the 19th Century by plant breeding, but little further improvement in concentration has been made since then as breeders have turned their attention to overall yield. There are reports of experimental breeding lines in which sucrose content is as high as 80% of the dry weight (24% of the fresh weight) (Jassem *et al*., 2000). However, this has been achieved at the expense of root yield, making these genotypes currently unacceptable for commercial production.

Sucrose content has been shown to be controlled by multiple genes (Jassem *et al*., 2000), and five quantitative trait loci (QTL) have been identified (Schneider *et al*., 2002). Clearly, genes that are likely to affect sucrose content directly are those in the sucrose biosynthetic pathway (Fig. 2), such as SPS, and those that break it down, namely sucrose synthase and the invertases, and all have been proposed to be important (Silvius & Snyder, 1979; Masuda *et al*., 1987; González *et al*., 2005). However, there are no reports of attempts to alter expression of genes encoding these enzymes by genetic modification and there is currently no use of genetic markers for these or other genes in sugar beet plant breeding. Instead, breeders base selections on sugar concentration measured on a fresh weight basis. Unfortunately, this is affected by environmental conditions, particularly drought (Ober *et al*., 2004), making interpretation difficult.

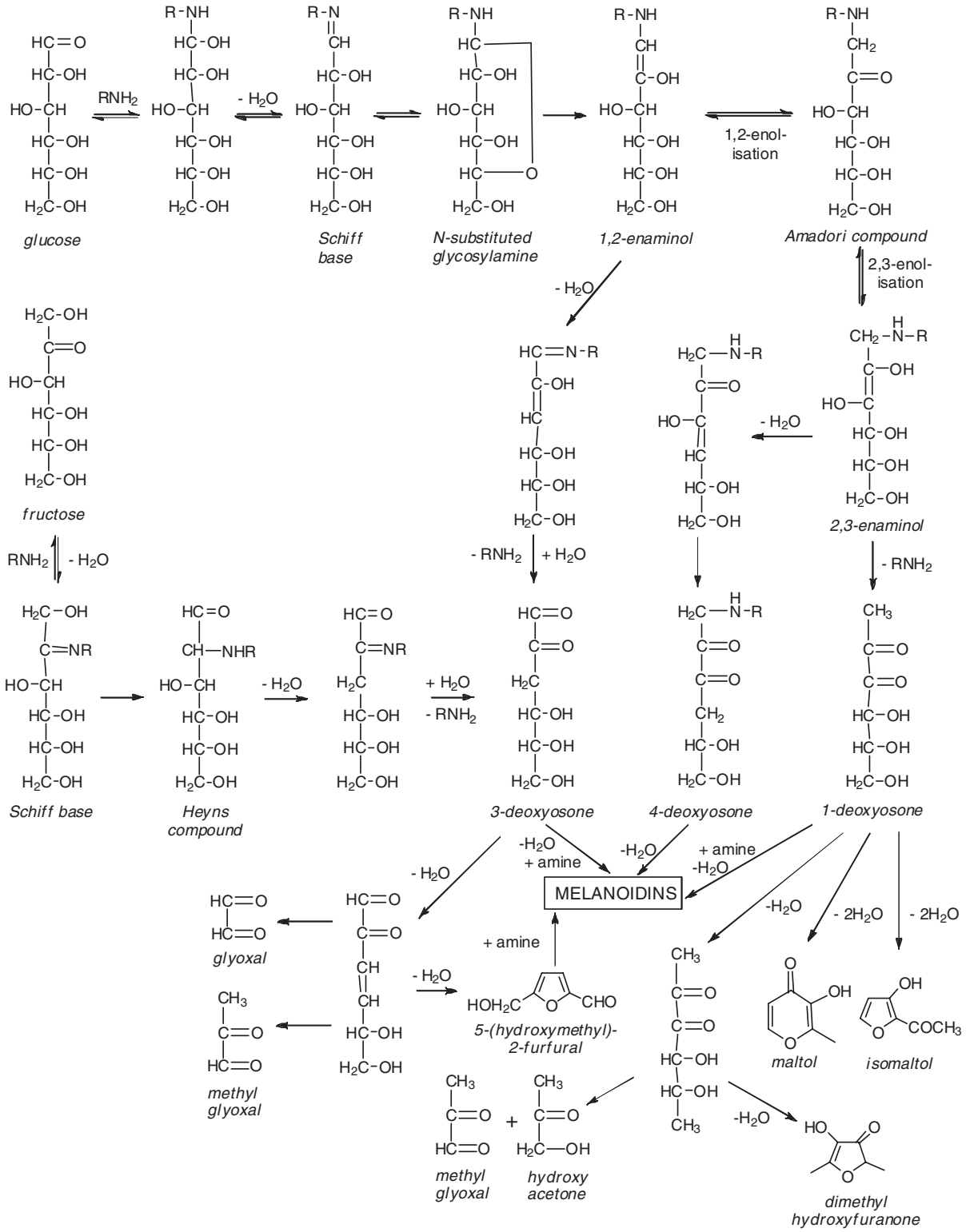

Figure 4 The Maillard reaction showing the formation of Amadori and Heyns intermediates and their breakdown to carbonyl compounds.

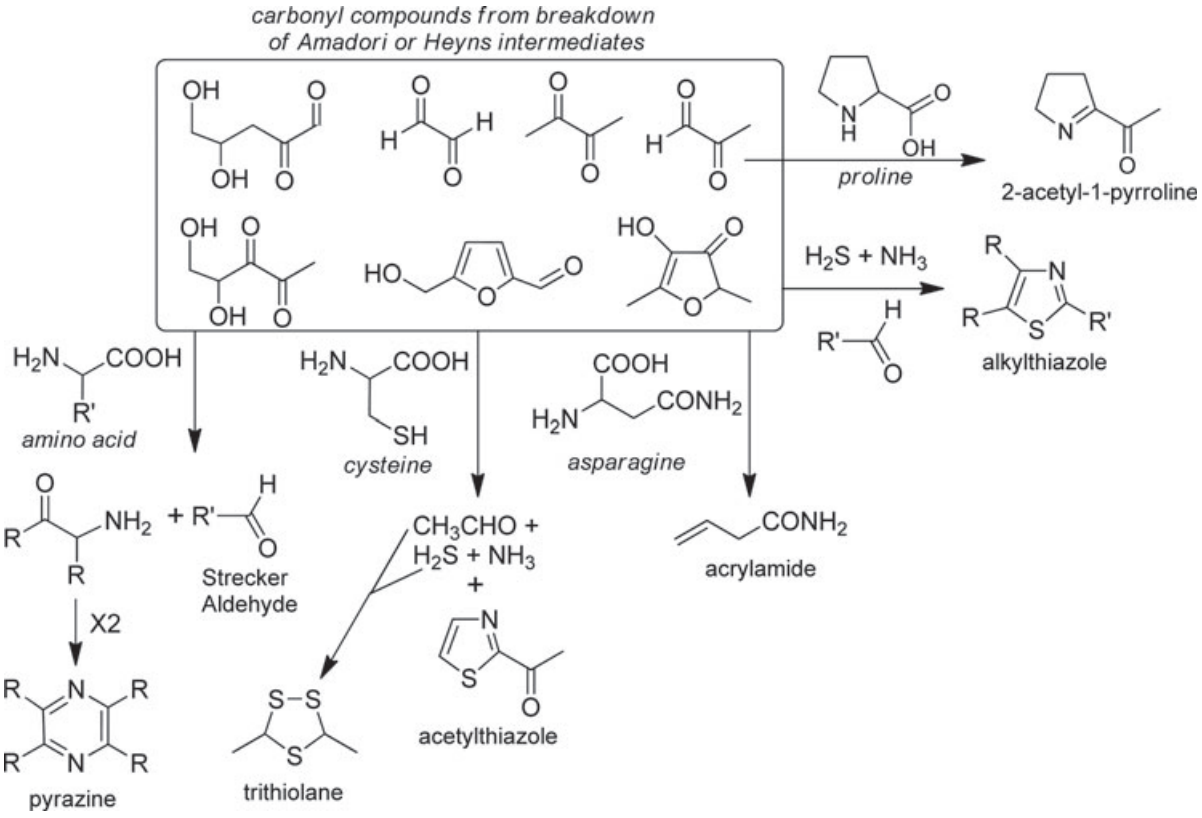

Figure 5 Scheme for the formation of some classes of aroma compounds and acrylamide in the later stages of the Maillard reaction.

In sugar cane, sucrose is harvested from the stems (culms) and can make up 20% of the dry weight in mature internodes. Sugarcane cultivars differ in their capacity to accumulate sucrose and in a recent study, 37 QTL associated with sucrose concentration were discovered (Aitken *et al*., 2006). In another study, the gene expression profiles of 30 genotypes were compared and related to sucrose content (Papini-Terzi *et al*., 2009). The results suggested that genes involved in drought stress responses and, to a lesser extent, ABA signalling and cell wall biosynthesis were important.

There has been some success in increasing total sugar content of sugar cane by introducing a bacterial sucrose isomerase gene through genetic modification (Wu & Birch, 2007). Most of the additional sugar in the genetically modified plants was in the form of isomaltulose (also known as palatinose), which is used as a sucrose substitute in some foods because it has a very sweet taste. It is fully digested by humans and has the same calorific value as sucrose, but it has a lower glycaemic index and unlike sucrose it does not cause dental caries.

In contrast to these crops, potatoes have been bred for low sugar content for many years in order to enable manufacturers to produce chips (French fries), crisps and related products with an even, light brown colour. This turned out to be fortuitous when the issue of acrylamide formation in fried, roasted and baked products arose in 2002. The relationship between sugars, free asparagine, other free amino acids and acrylamide formation in potato is complex: while some studies have reported the concentration of reducing sugars to be the main determinant of acrylamide levels (Amrein *et al*., 2003), others have found that the ratio of free asparagine to other amino acids is also important (Elmore *et al*., 2007; reviewed by Halford *et al*., 2007; Muttucumaru *et al*., 2008). The latter may result from competition between free amino acids for participation in the Maillard reaction when concentrations of sugars are limiting, which can occur in potato. Nevertheless, there are anecdotal reports that food processors have had some success in reducing acrylamide formation in their products by rejecting potatoes with high sugar content.

Despite the reductions in sugar concentration in potato tubers that have been achieved, there remains considerable variation between different potato genotypes (Amrein *et al*., 2003; Kumar *et al*., 2004; Elmore *et al*., 2007) and this affects processing properties. In the

study by Kumar *et al.* (2004), for example, tubers of good chipping varieties were found to contain 1.91 mg sucrose g^{-1} fresh weight, while those of poor varieties contained on average 4.53 mg g^{-1}. Pre- and postharvest environmental and management factors are also important, including temperature, mineral nutrition and water availability during cultivation, crop maturity at harvest, mechanical stress and storage conditions (Kumar *et al.*, 2004). Temperature during cultivation is a major factor because the processes of photosynthesis, transpiration, translocation of carbohydrates and respiration are all temperature dependent. The optimum temperature range for most varieties is quite narrow, between 15°C and 20°C (Kumar *et al.*, 2004).

There have been somewhat contradictory reports on the effect of soil nitrogen on sugar concentrations in potato tubers. De Wilde *et al.* (2006) showed that the levels of tuber sugars rose in nitrogen-deprived potatoes by up to 100% compared with adequately fertilised potatoes, and Kumar *et al.* (2004) similarly reported that plants adequately fertilised with N had lower reducing sugar concentration at harvest. In contrast, Amrein *et al.* (2003) found no correlation between the amount of nitrogen fertilization and acrylamide formation, which was assumed to be at least partly dependent on sugar concentration, in 57 samples of cooked, grated potato, from 10 different cultivars. The effect of sulphur deprivation has also been investigated and found to cause large increases in the levels of sugars (Elmore *et al.*, 2007). The three cultivars in that study, King Edward, Maris Piper and Prairie, all showed an increase in overall sugar concentration in response to sulphur deprivation, but there were significant differences between the varieties. Notably, the concentration of fructose more than doubled in Prairie tubers while glucose increased from 1.74 to 7.81 mmol kg^{-1} dry weight in Maris Piper tubers. In this study, the changes in sugar content of the tubers did not correlate with the amount of acrylamide that formed on heating, while free asparagine as a proportion of the total free amino acid pool did show a clear correlation. The concentrations of Strecker aldehydes and compounds formed from their condensation were also affected, as were a number of other volatiles that were formed on heating (Elmore *et al.*, 2010).

The other major factor affecting sugar content, and the one to which producers and processors pay most attention, is postharvest storage. Potato tubers are considered to be dormant, but they are much more active biochemically than cereal grain, for example, and, therefore, are much more sensitive to storage conditions. Sugars will accumulate rapidly in stored potato tubers in response to the temperature falling below approximately 8–10°C (cold sweetening; Sowokinos, 1990), sprouting (dormancy break) and tuber senescence after long-term storage. Storage atmosphere is also important because low oxygen levels suppress sugar accumulation at low temperature, while an increase in carbon dioxide concentration has the opposite effect (Kumar *et al.*, 2004).

Storage is particularly problematic because potato tubers have to be stored for long periods to enable a year-round supply for consumers and the food industry. The tubers are chilled to prevent sprouting but storage at 4°C, which is very effective at preventing sprouting, results in cold sweetening as starch is broken down and sugars accumulate. Starch degradation appears to be initiated primarily through the action of starch phosphorylase (Malone *et al.*, 2006; Morales *et al.*, 2008). Glucose and fructose will accumulate to such an extent that they cause localised blackening of crisps and chips during frying. It is now known, as we describe above, that sugar accumulation may also lead to an increase in the risk of acrylamide formation during processing. Tubers are therefore usually stored at higher temperatures, typically 8–10°C, to prevent cold sweetening, and processors go to great lengths to avoid any kind of temperature shock to the tubers, bringing them down to the long-term storage temperature in small steps over a period of several weeks. Sprouting, which would occur at this temperature, is controlled by spraying with chemical sprout suppressants such as chloropropham (CIPC). There are concerns regarding residues of these chemicals in the food product (Bradshaw, 2006) and the maximum residue limit for CIPC has been reduced recently to 10 ppm. Clearly, this is an intractable problem, made more acute by the discovery that sugars are precursors for acrylamide formation.

There has been some success in improving storage properties of potatoes using a transgenic approach (Rommens *et al.*, 2006). The expression of starch-associated R1 (an α-glucan, water dikinase) and starch phosphorylase-L (*PhL*) genes was suppressed in potatoes of variety Russet Burbank. This reduced cold- and stress-induced sweetening, enhanced fry aroma, increased starch content and reduced acrylamide formation during processing. In the same experiment, the expression of polyphenol oxidase (*Ppo*) was also suppressed, resulting in improved bruise resistance.

In contrast to the situation with potato, sugar concentration in cereal grain has not been an important target for breeders up to now. That may change as the acrylamide issue highlights the link between raw material composition and processing properties, both favourable and unfavourable. Sucrose is by far the major sugar in wheat grain but it is notable that the major reducing sugar is maltose (Table 1). Significant genotypic variation in grain sugar content has been reported in several studies

(Claus *et al.*, 2006; Muttucumaru *et al.*, 2006; Hamlet *et al.*, 2008). Environmental factors are also important. For example, high temperatures during grain filling have been reported to cause an increase in sucrose, reducing sugars and sugar phosphates, and a reduction in starch (Jenner, 1991). Gooding *et al.* (2003) also reported a decrease in Hagberg falling number, which is indicative of reduced starch and increased sugars, in heat-stressed wheat.

Perhaps surprisingly, sugar concentrations in the vegetative parts of the wheat plant have received considerably more attention from plant physiologists than those in the grain. For example, there has been considerable research on the effect of abiotic stresses on the sugar content of wheat leaves and seedlings. Osmotic stresses, including those caused by salt, freezing and drought, generally cause an increase in sugar content, with tolerant varieties accumulating higher concentrations than intolerant varieties (Sarker *et al.*, 1999; Sairam *et al.*, 2002; Nayyer & Walia, 2004; Guo *et al.*, 2009; Zheng *et al.*, 2009). This is an example of how plants control the partitioning between soluble and insoluble carbohydrates for osmoregulation. Sugars have also been shown to accumulate in response to hypoxia and early senescence (Mustroph & Albrecht, 2007; Caid *et al.*, 2008). Long-term exposure of wheat plants to salt stress has also been shown to cause an increase in fructan accumulation (Kerepesi *et al.*, 2000), which, as we describe above, is a characteristic generally associated with drought and osmotic stress tolerance in plants.

Seeding date of winter wheat has also been shown to affect the accumulation of soluble carbohydrates and fructan in vegetative wheat tissues (Gaudet *et al.*, 2001). Early planting generally results in lower sugar and higher fructan levels in the autumn and winter, and this is associated with improved resistance to fungal infection.

Sugar concentrations in rye and barley are affected by similar factors to those affecting sugar concentrations in wheat. In barley these factors include genotype, salt stress, nitrogen supply, abiotic stresses such as salt, water, cold and osmotic stress, aphid infestation and heavy metal toxicity (aluminium, copper and cadmium) (Zúniga *et al.*, 1990; Cabrera *et al.*, 1994; Murelli *et al.*, 1995; Olien & Clark, 1995; Teulat *et al.*, 1997; Amer, 1999; Guo *et al.*, 2007; Widodo *et al.*, 2009). As with wheat, the focus of most of the research has been the vegetative parts of the plant. The accumulation of soluble sugars has been shown to be associated with osmotic adjustment and has been suggested as a criterion for rapid evaluation of osmotic adjustment capacity in segregating populations, along with leaf osmotic potential and relative water content (Teulat *et al.*, 1997). The concentration of reducing sugars is affected by osmotic stress during grain development mainly as a result of enhanced β-amylase activity; this also affects source–sink interactions (Wei *et al.*, 2009). Salt stress causes an increase in reducing sugars and a decrease in non-reducing sugars, possibly associated with osmotic adjustment (Amer, 1999). The capacity for osmotic adjustment and therefore salt tolerance is enhanced by elevated CO_2, possibly because of the energy requirement of the process, which involves uptake and accumulation of ions as well as modulation of sugar levels and other osmolytes (Pérez-López *et al.*, 2010).

In rye, the factors known to affect the concentration of reducing sugars include variety, cold stress, cold acclimation, freezing, heat shock, salt stress and sowing date. Sugars act as osmoprotectants and interact with the lipid bilayer during cold stress and freezing (Antikainen & Pihakaski, 1994; Ma *et al.*, 2009). In one study, total soluble sugar content was shown to double by the second week of cold acclimation (Koster & Lynch, 1992), while in another, carbohydrates (sucrose, fructose, glucose and fructan) were shown to account for approximately 60% of the dry weight after 3 weeks of cold acclimation at 3°C (Livingston *et al.*, 2006). Fructan accumulation also plays a role in dehydration tolerance and has been shown to improve rye liposome stability during drying and rehydration (Hincha *et al.*, 2007). The simple sugars and long-chain carbohydrate polymers, including fructan, as well as the proteins that accumulate during cold acclimation, also improve heat shock tolerance (Fu *et al.*, 1998).

Although rye responds to stresses in a similar way to wheat and barley, it has superior freezing tolerance to either of its close relatives, possibly associated with greater capacity for carbohydrate storage (Trunova *et al.*, 1997) and greater hydrolysis of fructan to increase concentrations of simple sugars to relieve critical freezing stress (Olien & Clark, 1995).

Rice is distinct from the other cereals and most other land plants in that it has the ability to germinate and grow while submerged (Perata *et al.*, 1997). This characteristic is crucial given that rice is often cultivated in areas that are prone to flooding. The ability to tolerate anaerobic conditions requires that sufficient energy is generated by the inefficient process of fermentation (Webb & Armstrong, 1983; Ricard *et al.*, 1991). This requires mobilization of starch in the endosperm to provide sugars, and rice, unlike the other cereals, is able to do this under anaerobic conditions (Guglielminetti *et al.*, 1995*a*, *b*). This appears to be at least in part because sugar inhibition of gene expression of α-amylases of the Amy3 subfamily is suspended during anaerobic germination, enabling high concentrations of sugars to accumulate for fermentation without inhibiting starch breakdown (Hwang *et al.*, 1999).

The ability of rice to germinate in the presence of salt is also associated with α-amylase activity and the maintenance of soluble sugar concentrations (Liu *et al.*, 2007), and salt stress has also been shown to reduce soluble sugar levels in rice leaves (Sultana *et al.*, 1999). Mild water deficit, on the other hand, causes an accumulation of sugars in the leaf sheath (Cabuslay *et al.*, 2002) and affects enzymes involved in the conversion of sucrose to starch, with a concomitant effect on grain filling (Yang *et al.*, 2003). Sucrose synthase activity, for example, has been shown to be substantially enhanced by mild water stress and to correlate with starch accumulation in the grain (Yang *et al.*, 2003). Nitrogen fertilisation also affects sugar concentrations (Wang *et al.*, 2006).

The factors that have been shown to affect sugar content in maize include genotype, temperature, flooding, salt stress, drought, exposure to selenium, osmotic stress and UV-B radiation (Zaidi *et al.*, 2003; Kwabiah, 2004; Quaggiotti *et al.*, 2004; Ogawa & Yamauchi, 2006; Boomsma & Vyn, 2008; Kruse *et al.*, 2008; Shen *et al.*, 2008; Hajlaoui *et al.*, 2009; Suwa *et al.*, 2010). Varieties of maize that accumulate very high concentrations of sugars in the kernels are used to produce sweetcorn. These varieties were developed from genotypes with naturally occurring mutations in one or more genes encoding enzymes in the starch biosynthesis pathway, so starch biosynthesis is impaired. The mutated genes include *sugary-1* (*Su1*), which encodes a starch-debranching enzyme (isoamylase-1), *sugary enhancer-1* (*Se1*), which acts in combination with the homozygous mutant *su1* allele to increase sugar levels further, *shrunken-2* (*Sh2*) and *Brittle-2* (*Bt2*), which, as we have said, encode subunits of ADP-glucose pyrophosphorylase, and more. Sweetcorn is harvested when still immature, otherwise the kernels become tough and starchy.

Concluding remarks

We have reviewed the most abundant sugars in the world's most important crop plants, from the production of sucrose as the primary product of photosynthesis and major carbon transport molecule, through primary metabolism and sugar signalling to the synthesis and breakdown of storage carbohydrates. We have emphasised the importance of sugars to the processing properties of potato and cereals, in particular their participation in the Maillard reaction to produce flavours, aromas and colours, and potentially harmful compounds such as acrylamide. This aspect of sugars in crop plants has perhaps not yet attracted the attention of crop physiologists, breeders and biotechnologists in the way that one might expect. It is notable, for example, that most of the studies on the factors that affect sugar accumulation in cereals have focused on the vegetative parts of the plant. This may change as food processors become more aware of the effect of raw material composition on their products, partly as a result of the acrylamide issue, and demand varieties that are tailored for specific end uses.

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